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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

BLUNDELL et al.

Serial No. **09/820,745**

Filed: **March 30, 2001**

For: **CRYSTAL STRUCTURE**

Atty. Ref.: **620-139**

Group: **1651**

Examiner:

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November 19, 2001

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

AMENDMENT

Responsive to the Notice to Comply dated September 18, 2001 (copy attached), entry and consideration of the following amendments and remarks are requested.

IN THE SPECIFICATION

Amend the specification as follows.

Pages 20-21, delete the paragraph beginning on page 20, line 19 and extending through to page 21, line 6, and insert the following therefor:

--35 proteins or translated gene-sequences have been identified using a PSI-BLAST search, with high enough similarity to be classified as members of the KPHMT family (22) . The enzyme is found in bacteria, lower eukaryotes (e.g. yeast) and in the plant *Arabidopsis thaliana* but is not found in *Caenorhabditis elegans, Drosophila melanogaster* or, as yet, in other higher eukaryotes. This is consistent with the end product of this pathway being a vitamin. We have analyzed the sequences from the 35

members of this family to identify residues important to the mode of action. Correlation between primary structure among five members of the KPHMT family and the secondary structure of the *E. coli* enzyme is shown in Figure 5 (SEQ ID NOs:7-12, respectively). The consensus sequence, generated by ClustalW (23) with the sequences of the 35 members, highlights that of the 264 residues, 23 residues are invariant while an additional 77 are conserved. Six conserved sequence motifs, at least six residues in length, were also identified. These are ^{42}LeuValGlyAspSerLeuGlyMet^{49} (SEQ ID NO:1), ^{111}ValLysIleGluGlyGly^{116} (SEQ ID NO:2), ^{135}GlyHisXGlyLeuThrProGln^{142} (SEQ ID NO:3) (where X is a hydrophobic residue) , ^{148}GlyGlyTyrLysValGlnGly^{154} (SEQ ID NO:4), ^{200}IleGlyIleGlyAlaGly^{205} (SEQ ID NO:5) and ^{209}AspGlyAsnIleLeuVal^{214} (SEQ ID NO:6). The first two of the six motifs contain residues shown in the crystal structure to be involved in binding the ketopantoate (and hence the substrate) or metal ion.--

Insert the attached Sequence Listing after the claims pages.

REMARKS

Reconsideration is requested.

The specification has been amended to include the attached Sequence Listing and corresponding sequence identifiers. No new matter has been added. The attached paper and computer-readable copies of the Sequence Listing are the same. No new matter has been added. A separate Statement to this effect is attached.

The above and attached is believed to be fully responsive to the Notice to Comply dated September 18, 2001, however the Office is requested to contact the undersigned if anything further is required in this regard.

An early and favorable Action on the merits of the claimed invention is requested.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____

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MARKED UP SPECIFICATION

Pages 20-21, delete the paragraph beginning on page 20, line 19 and extending through to page 21, line 6, and insert the following therefor:

--35 proteins or translated gene-sequences have been identified using a PSI-BLAST search, with high enough similarity to be classified as members of the KPHMT family (22) . The enzyme is found in bacteria, lower eukaryotes (e.g. yeast) and in the plant *Arabidopsis thaliana* but is not found in *Caenorhabditis elegans, Drosophila melanogaster* or, as yet, in other higher eukaryotes. This is consistent with the end product of this pathway being a vitamin. We have analyzed the sequences from the 35 members of this family to identify residues important to the mode of action. Correlation between primary structure among five members of the KPHMT family and the secondary structure of the *E. coli* enzyme is shown in Figure 5 (SEQ ID NOs:7-12, respectively). The consensus sequence, generated by ClustalW (23) with the sequences of the 35 members, highlights that of the 264 residues, 23 residues are invariant while an additional 77 are conserved. Six conserved sequence motifs, at least six residues in length, were also identified. These are ^{42}LeuValGlyAspSerLeuGlyMet^{49} (SEQ ID NO:1), ^{111}ValLysIleGluGlyGly^{116} (SEQ ID NO:2), ^{135}GlyHisXGlyLeuThrProGln^{142} (SEQ ID NO:3) (where X is a hydrophobic residue) , ^{148}GlyGlyTyrLysValGlnGly^{154} (SEQ ID NO:4), ^{200}IleGlyIleGlyAlaGly^{205} (SEQ ID NO:5) and ^{209}AspGlyAsnIleLeuVal^{214} (SEQ ID NO:6). The first two of the six motifs contain residues shown in the crystal structure to be involved in binding the ketopantoate (and hence the substrate) or metal ion.--